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The Local Effects of Topically Applied Estradiol, Cyproterone Acetate, and Ethanol on Sebaceous Secretion in Intact Male Rats

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Estradiol in ethanol was applied once daily to one flank of intact male rats and sebum production on both flanks was measured over periods of 18 h alternating with 6 h by absorbing the lipid on pads of cigarette paper held in place by a harness, starting on the 15th day. Sebum production was very significantly less on the treated flanks than on the contralateral flanks that received only vehicle, indicating an unequivocal local effect of the estradiol. At the same time, the values on the contralateral flanks were significantly below those of littermate rats which received vehicle only, indicating that the estrogen had been systemically absorbed to produce a distal action. The estradiol also significantly reduced plasma testosterone and the relative weights of the seminal vesicles, ventral prostate, and preputial glands, which demonstrated that part of the general action of the estrogen could have been by suppression of endogenous androgen production.

In short-term experiments, in which measurements were started concurrently with treatment, the inhibitory action of estradiol, in contrast to that of cyproterone acetate, was not detected until the 4th day. Indeed, it appeared probable that the estrogen actually stimulated sebum secretion over the first 2 days, suggesting that it had a biphasic effect.

That systemically administered estrogens depress sebaceous activity in humans and experimental animals is unquestionable. It is, however, debatable whether their action is peripheral and local or by way of some systemic mechanism. The facts surrounding the controversy have been fully explicated elsewhere [1,2] and only the main evidence requires recapitulation.

Because of their failure, even with the minimum effective dose, to elicit a local as distinct from a contralateral suppression of sebum secretion when ethinylestradiol was applied to one side of the forehead, Strauss, Kligman, and Pochi [3] originated the view that its action was not at the peripheral level. Strauss and Pochi [4] later suggested that the action could be by suppression of gonadotropins and hence of endogenous androgen production, a view supported by the evidence that sebum-suppressing doses of estrogen appear to reduce the levels of testosterone in plasma and urine [5].

The contrary view that the action of estrogens on sebaceous activity is direct and peripheral receives support from the fact that the suppressing effect can be demonstrated in rats against stimulation by exogenous androgen [6,7]. In addition, estrogens do not appear to act by reducing cell division, which should occur if endogenous androgen production were suppressed, since a major effect of androgens is to stimulate sebaceous mitosis [8,9].

The development of a method for measurement of sebum production in defined areas [10] has made possible a direct examination of the problem.

MATERIALS AND METHODS

Animals

Intact male albino rats from the randomly mated colony maintained in the Zoology Department of Sheffield University were used. Rat cake (Labsure PMD Nuts) and water were available ad libitum.

Design of Experiments

Long-term effects of estradiol and ethanol: Twenty-four rats, made up of 12 litters of 2 rats each were used for experiment at 19–26 weeks of age. One rat (I) of each litter was given ethanol, once daily, on one flank only and a second rat (II) received estradiol on one flank and ethanol on the other. Treatment was continued for 18 days and measurements of sebum production were made from days 15–18 of treatment, when the rats were killed.

Short-term effects of estradiol, cyproterone acetate, and ethanol: Thirty-six rats, made up of 12 litters of 3 rats each, were used for experiment at 20–31 weeks of age, when their body weights were 250–300 g. One rat (III) of each litter was given ethanol once daily, on one flank only, at 1500 h on the first day and at 0900 h on each succeeding day, a second rat (IV) received cyproterone acetate in ethanol on one flank and ethanol on the other flank, and a third rat (V) estradiol on one flank and ethanol on the other flank.

Sequential measurements of sebum production on both flanks were made over the first 4 days of treatment and the rats were killed on the 4th day.

Topical Administrations

Areas 45 × 22.5 mm were delimited on each flank by tattoo marks 3 days before the first treatment. Ethanol (0.2 ml), 5 mg cyproterone acetate in 0.2 ml ethanol, or 2 μg estradiol-17β were applied from a syringe, and the areas immediately dried with a hair dryer.

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Sebum Production

Lipid was gravimetrically estimated after absorbing it by pads of cigarette papers placed on the 2 areas, one on each flank, for each of 8 consecutive periods of 18 h alternating with 6 h, starting at 1500 h. The method has been previously described in detail [10].

Organ Weights and Estimation of Plasma Testosterone

At killing, blood was removed by a syringe from the ventricle while the rats were under anesthesia. Preputial glands, seminal vesicles, ventral prostates, and testes were dissected out and weighed fresh after blotting and, where appropriate, expressing contents.

For assay of testosterone, 100 μl plasma was extracted twice with 200 μl distilled ethanol and 100 μl of this extract used for radioimmunoassay using the procedure described by Kime and Manning [11].

RESULTS

Long-Term Effects of Estradiol

The lipid production during each of 4 successive 24-h periods for days 15–18 of treatment is shown in Table I.

That ethanol by itself causes a small but significant reduction in sebum production has already been noted and discussed elsewhere [10].

Estradiol unequivocally reduced sebaceous secretion. When the treated sites are compared with sites in control rats receiving only vehicle, the amounts were lowered to 40–50%. A two-tailed *t*-test (Table II, column a) shows the differences to be highly significant on each of the 4 days over which measurements were made.

To determine the extent to which the effect was produced locally, as distinct from generally by systemic absorption of the estrogen, it is necessary to compare the treated site with the contralateral flank of the treated rats. For this comparison, too, the differences were highly significant on each of days 15–18 (Table II, column c).

Finally, to determine how much of the total effect was produced by systemic absorption, as distinct from locally, the lipid production on the contralateral flanks of the estradiol-treated rats may be compared with that on the ethanol-treated flanks of the control rats. Though there was no significant difference on day 15, the differences on days 16, 17, and 18 were highly significant (Table II, column b). This demonstration does not, of course, prove that part of the effect of the estrogen was indirect, only that sufficient hormone had been taken into the circulation to affect the distal site. Whether or not part or all of this systemic effect might involve suppression of endogenous androgen production requires direct evidence of the androgen status of the treated rats. Such evidence comes from examination of the levels of plasma testosterone and the relative weights of androgen-sensitive and other organs, shown in Table III.

It is evident that plasma testosterone was markedly and significantly reduced by the estrogen treatment. Moreover, this lowering of available androgen was reflected in the significantly lower weights of the seminal vesicles, ventral prostate, and preputial glands. However, there are no significant differences in testis, pituitary, or body weight.

It may be concluded that estradiol applied topically acts locally and peripherally to reduce sebum production, but that sufficient estradiol is systemically absorbed to produce lesser effects at distal sites. That part of the effect could be by suppression of endogenous androgen secretion is suggested by the reduction in plasma testosterone and the relative weights of the seminal vesicles, ventral prostate, and preputial glands.

Short-Term Effects of Estradiol and of Cyproterone Acetate

Having established that estradiol reduces sebaceous secretion in the long term, a major question is whether the effect is initiated immediately upon treatment, as previously shown for cyproterone acetate in androgen-treated rats [10] or after a delay in which stimulation may have occurred, as suggested by an earlier study [12].

The lipid production during each of 4 24-h periods on each of the variously treated sites is shown in Table IV.

The analysis of the results is complicated by the fact that there are clearly sequential changes within each group as well as differences due to the treatments. Thus the means are in general lower on day 2 than day 1 and higher on day 4 than day 3. Higher levels during the first 24 h of measurement are characteristic of the method and almost certainly result from the absorption of existing surface lipid as distinct from newly secreted sebum. Further, if the hair on the flanks is growing during any experimental period, absorption of the secreted sebum by the test papers may be incomplete; if the hair is then shaved, there will be a compensatory increase in the lipid picked up during the ensuing period of measurement. This could have accounted for the higher means on day 4.

These considerations do not invalidate the use of the two-tailed *t*-test for making lateral comparisons. However, the data have also been subjected to profile analysis [13].

The effect of cyproterone acetate may be examined by com-

TABLE I. *Lipid production in estradiol treatment*

Group	I		II	
Topical treatment	None	Ethanol	Ethanol + estradiol	Ethanol
Day 15	2.59 ± 0.206	2.20 ± 0.152	1.68 ± 0.148	2.30 ± 0.180
Day 16	1.72 ± 0.130	1.56 ± 0.067	0.79 ± 0.058	1.16 ± 0.078
Day 17	1.70 ± 0.084	1.50 ± 0.061	0.83 ± 0.045	1.20 ± 0.088
Day 18	1.46 ± 0.105	1.36 ± 0.086	0.81 ± 0.047	1.07 ± 0.062

Each figure is the sum of 2 successive measurements over periods of 18 and 6 h, respectively. Intact male rats in littermate pairs were used. Simultaneous measurements were made on each of the 2 flanks, which received topical applications as shown. Means ± SE for groups of 12.

TABLE III. *Plasma testosterone, organ weights per 100 g body weight, and body weights of untreated intact male rats and of rats receiving topical estradiol daily for 18 days*

	Control	Estradiol treated	t	*p*
Plasma testosterone (ng per ml)	1.26 ± 0.21*	0.60 ± 0.07*	3.0	<0.01
Testes (g)	1.03 ± 0.025*	1.01 ± 0.034*	0.5	NS
Seminal vesicles (mg)	166 ± 14.5	102 ± 9.4	3.7	<0.002
Ventral prostate (mg)	85.6 ± 7.0	54.7 ± 6.1	3.3	<0.01
Preputial glands (mg)	26.4 ± 2.1	19.7 ± 1.5	2.6	<0.02
Pituitary (mg)	3.88 ± 0.59*	3.93 ± 0.14*	0.1	NS
Body weight (g)	247 ± 13	236 ± 9	0.7	NS

Means ± SE for groups of 12 (*9 only).

TABLE II. *Long-term effect of estradiol.*

Day	(a) Estradiol in treated rats vs vehicle in control rats			(b) Vehicle in treated rats vs vehicle in control rats			*(c) Estradiol vs vehicle in treated rats*		
	Difference	t	*p*	Difference	t	*p*	Difference	t	*p*
15	−0.52 ± 0.180	2.9	<0.02	+0.10 ± 0.166	0.6	NS	−0.62 ± 0.156	4.0	<0.01
16	−0.78 ± 0.098	7.9	<0.001	−0.41 ± 0.121	3.3	<0.01	−0.37 ± 0.065	5.8	<0.001
17	−0.67 ± 0.073	9.2	<0.001	−0.30 ± 0.118	2.6	<0.05	−0.37 ± 0.090	4.1	<0.002
18	−0.54 ± 0.080	6.7	<0.001	−0.29 ± 0.079	3.7	<0.01	−0.26 ± 0.050	5.2	<0.001

Mean differences in sebum production (mg/10 cm^2/24 h) between sites and animals as shown. Twelve pairs in each comparison.

TABLE IV. *Lipid production in mg per 10 cm² of body surface on each of 4 successive periods of 24 h, starting at 15 h*

Group	III		IV		V	
Topical treatment	None	Ethanol	Ethanol + cyproterone acetate	Ethanol	Ethanol + estradiol	Ethanol
Day 1	1.65 ± 0.125*	1.51 ± 0.111	1.59 ± 0.146	1.61 ± 0.075	1.73 ± 0.110	1.67 ± 0.108
Day 2	1.48 ± 0.082	1.20 ± 0.064	1.24 ± 0.104	1.32 ± 0.066	1.47 ± 0.091	1.37 ± 0.073
Day 3	1.63 ± 0.091	1.28 ± 0.090	1.20 ± 0.100	1.38 ± 0.079	1.38 ± 0.079	1.36 ± 0.080
Day 4	1.83 ± 0.190	1.53 ± 0.136	1.41 ± 0.170	1.59 ± 0.167*	1.57 ± 0.167	1.71 ± 0.173

Intact male rats in litters of 3 were used. Mean ± SE for groups of 12 (*11 only). Other details as for Table I.

TABLE V. *Short-term effect of cyproterone acetate*

Day	(a) Cyproterone acetate in treated rats vs vehicle in control rats			(b) Vehicle in treated rats vs vehicle in control rats			(c) Cyproterone acetate vs vehicle in treated rats		
	Difference	t	p	Difference	t	p	Difference	t	p
1	+0.07 ± 0.103	0.6	NS	+0.09 ± 0.072	1.3	NS	−0.02 ± 0.106	0.2	NS
2	+0.03 ± 0.067	0.5	NS	+0.12 ± 0.048	2.4	<0.05	−0.08 ± 0.065	1.2	NS
3	−0.09 ± 0.085	1.0	NS	+0.10 ± 0.070	1.4	NS	−0.18 ± 0.053	3.5	<0.01
4	−0.12 ± 0.064	1.9	<0.10	+0.02 ± 0.067*	0.3	NS	−0.15 ± 0.064*	2.3	<0.05

Mean differences in sebum production (mg/10 cm²/24 h) between sites and animals as shown. Twelve (*11 only) pairs in each comparison.

TABLE VI. *Short-term effect of estradiol*

Day	(a) Estradiol in treated rats vs vehicle in control rats			(b) Vehicle in treated rats vs vehicle in control rats			(c) Estradiol vs vehicle in treated rats		
	Difference	t	p	Difference	t	p	Difference	t	p
1	+0.22 ± 0.100	2.2	<0.06	+0.16 ± 0.113	1.4	NS	+0.06 ± 0.108	0.5	NS
2	+0.27 ± 0.079	3.4	<0.01	+0.17 ± 0.053	3.1	<0.01	+0.11 ± 0.076	1.4	NS
3	+0.09 ± 0.081	1.2	NS	+0.08 ± 0.084	0.9	NS	+0.02 ± 0.034	0.5	NS
4	+0.04 ± 0.074	0.5	NS	+0.18 ± 0.064	2.8	<0.02	−0.14 ± 0.049	2.9	<0.02

Mean differences in sebum production (mg/10 cm²/24 h) between sites and animals as shown. Twelve pairs in each comparison.

TABLE VII. *Organ weights per 100 g body weight of untreated intact male rats and of rats receiving topical cyproterone acetate or estradiol daily for 4 days*

	Control	Cyproterone acetate in ethanol	t	p	Estradiol in ethanol	t	p
Seminal vesicles (mg)	143 ± 7.0	149 ± 12.6	0.4	NS	127 ± 6.1	1.7	NS
Ventral prostate (mg)	84.6 ± 4.3	95.9 ± 6.5	1.4	NS	93.5 ± 4.4	1.4	NS
Preputial glands (mg)	32.5 ± 2.2	33.0 ± 2.6	0.1	NS	31.1 ± 3.6	0.3	NS

Means ± SE for groups of 12.

paring the treated with the contralateral flanks. The profiles appeared parallel, but their mean heights were not constant from day to day. The mean profile heights were not significantly different between treated and untreated, but the mean values for the treated flanks were significantly less than those for the untreated flanks on each of days 3 and 4 (Table V, column c). This reduction due to cyproterone acetate appeared to be entirely local, since the values for the contralateral flanks did not differ significantly from those for the flanks of control littermates treated with vehicle alone (Table V, column b).

The effect of estradiol can similarly be examined by comparing the treated with the contralateral flanks. The mean profile heights did not significantly differ between flanks, but did significantly change from day to day. On the estradiol-treated sites lipid production was significantly less on day 4, but appeared to be slightly, though not significantly, more on each of the 3 preceding days (Table VI, column c).

This suggestion that inhibition by estradiol may be preceded by a period of stimulation receives good support from a comparison of the treated flanks with those of littermates given vehicle only. Here profile analysis suggests that the mean profile heights may differ, though only at the 0.1 level of significance. The major factor contributing to this difference is the higher value on the estradiol-treated flanks on the first 2 days of treatment, each of which was statistically significant (Table VI, column a).

In the short term, neither cyproterone acetate nor estradiol caused any significant changes in the relative weights of the seminal vesicles, ventral prostate, or preputial glands (Table VII).

In conclusion, the evidence suggests that inhibition of sebum secretion by topically applied estradiol only starts to become manifest by the fourth day, and is preceded by a phase of stimulation during the initial 2 days. These effects do not appear to involve any systemic mechanism. No such biphasic effect occurs in the action of cyproterone acetate.

DISCUSSION

The results demonstrate unequivocally that estradiol applied topically in ethanol once daily for a period of 15 days reduced the sebum production on the flanks of intact male rats by a local action. A somewhat less marked, but nevertheless highly significant, reduction was produced on the contralateral flank, indicating that the applied hormone was also taken up into the circulation. The finding that in the estrogen-treated rats the plasma testosterone and the relative weights of the seminal vesicles, ventral prostate, and preputial glands were all significantly reduced indicates, but does not prove, that part of the general effect could have been due to an indirect systemic action of the estradiol in reducing androgen production. Thus, the experiments, on the one hand, establish with certainty that estrogen has a peripheral action on the sebaceous glands and, on the other hand, indicate the possibility that it could also affect them by an indirect systemic route.

The inhibitory action of estradiol did not appear to start immediately on application; the first signs of inhibition did not occur until the 4th day. Indeed, the immediate action of the estrogen appeared to be one of stimulation. This is clearly evident from the comparison (Table VI, column a) of the estradiol-treated sites with those in untreated littermates given vehicle only. The smaller and statistically insignificant difference between treated and untreated flanks in the treated animals (Table VI, column c) may be explained on the assumption that sufficient estradiol is quickly absorbed to effect the contralateral flank as well as the treated area. This explanation is borne out by the finding that by day 2 the sebum production on the untreated flanks was very significantly more than that on the untreated littermates (Table VI, column b).

That the action of estrogen on sebaceous activity might be biphasic was first suggested by Ebling in 1951 [12]. Using a histologic method, he assessed changes in the size of the sebaceous glands in skin samples taken sequentially at biopsy from immature female rats following subcutaneous implantation of estradiol benzoate crystals. The glands increased in size for 2 days, and then decreased progressively on days 3, 5, and 9 to a level much below normal on day 20. The findings were exactly in parallel with the events of the current study.

These facts support the view of Bullough [14] that in a number of tissues estrogen can, over a short term, act as a stimulator of mitosis. According to Bullough, the incidence of mitoses in the sebaceous glands of the female mouse fluctuates with the estrous cycle, reaching its peak in early estrus. Moreover, the injection of estrone in early diestrus is followed by a peak in sebaceous mitoses in 2 days.

Ebling [15] believed there were changes in the size of the rat sebaceous glands during the estrous cycle, with the peak at proestrus, but was unable to detect significant changes in the mitotic rate.

The evidence strongly suggests that estrogens must have more than one point of action. If, as seems probable, there is an initial stimulus to mitosis, this is soon overtaken by inhibition of sebaceous activity at another level, possibly, for example, by interference with intracellular lipid synthesis.

How is the suppressive action of the estrogen brought about? Sansone-Bazzano, Reisner, and Bazzano [16] suggested that estradiol might suppress the uptake of testosterone and its metabolism to 5α-dihydrotestosterone. Their evidence came from incubation of preputial glands from rats pretreated with estradiol for 2 weeks. However, they used doses (250 μg/day) about 100 times those shown by Ebling [9] to bring about marked inhibition of sebaceous secretion without suppressing the in vivo uptake by skin of radioactive testosterone [1]. Additional evidence against the view that estrogens act in a similar way to antiandrogens is that, if this were true, it might be expected that they would suppress cell division. In fact, estradiol reduces sebaceous gland size even in rats in which mitosis remains stimulated by exogenous testosterone [8,9]. Moreover, whereas the antiandrogens 17α-B-nortestosterone [6] and cyproterone acetate [7] reduce both sebum secretion and sebaceous mitosis in testosterone-stimulated rats, systemically administered estradiol appears to have a profound effect on secretion without any comparable effect on mitosis. Finally, it may be noted that, while much of the effect of testosterone in stimulating sebaceous secretion in the rat is dependent upon the presence of the pituitary [8,17,18], the suppressing effect of estradiol is not [19]. For all these reasons, it seems that the actions of estrogens must be clearly distinguished from those of antiandrogens.

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